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SUMMARY: SAFETY AND EFFECTIVENESS INFORMATION FOR CEP X SpectrumOrange/CEP Y SpectrumGreen DNA Probe Kit

The CEP X/Y probe is a combination of CEP X SpectrumOrange and CEP Y SpectrumGreen fluorescently labeled DNA probes for the alpha DNA on the centromeric region of chromosome X and the satellite III DNA at the Yq12 region of chromosome Y. This assay is designed to provide a reliable method for the simultaneous detection and enumeration of chromosomes X and Y in both interphase nuclei and metaphase spreads in bone marrow by fluorescence *in situ* hybridization (FISH).

Standard cytogenetic analysis detects the presence of the X and Y chromosomes by karyotyping metaphase spreads after staining the chromosomes with a dye in cultured tissue cells.

Safety and effectiveness issues relevant to FISH assays such as the CEP X/Y assay may include cross-reactivity, poor sensitivity, poor specificity, or poor reproducibility.

Analytical Sensitivity and Specificity

Hybridization Efficiency

In a pivotal study, the average percentage of cells with only one hybridization signal was 0.012% (S.D.=0.15%) on 143 bone marrow specimens. Thus, <2% cells with only one signal is a realistic standard of acceptance.

Analytical Sensitivity

The analytical sensitivity of the CEP X/Y probe was tested in the reproducibility study described below. In that study, the 0% XY specimen was estimated with a mean of 0.00% (s.d.=0.00%) XY nuclei and the 1% XY specimen, 0.94% (s.d.=0.32%). The 0% XX specimen was estimated with a mean of 0.00% (s.d.=0.00%) XX nuclei and the 1% XX specimen, 0.95% (s.d.=0.34%). There was little overlap between the 0% and 1% specimens; the lower 95% confidence limit for the 1% specimen was 0.31% and 0.28% for XY and XX, respectively. Thus, the limit of detection for CEP X/Y is estimated to be 1.0%.

Analytical Specificity

Locus specificity studies were performed with metaphase spreads according to standard Vysis QC protocols. A total of 65 metaphase spreads were examined sequentially by G-banding to identify chromosomes X and Y, followed by FISH. No cross-hybridization to other chromosome loci was observed in any of the 65 cells examined; hybridization was limited to the centromere of chromosome X and the Yq12 region of chromosome Y.

Reproducibility

To assess the reproducibility of the CEP X/Y interphase analysis for the percentage of cells with XX and XY signals, bone marrow specimens with approximately 0%/100%, 1%/99%, 5%/95%, 95%/5% XY/XX, 99%/1% and 100%/0% XY/XX were prepared. Inter-site, inter-lot, inter-day, and inter-observer reproducibility were assessed in a pivotal study with two of these bone marrow specimen mixtures (approximately 99%/1% and 100%/0% XY/XX,) and two mixtures of hematologically derived human cells with approximately 0%/100% and 1%/99% XY/XX. The percentage of cells with XX and XY signals were evaluated according to the instructions for signal enumeration in the package insert. Using ANOVA, significant, site-to-site and observer-to-observer variations were observed, reflecting the subjectivity of the visual enumeration process. In addition to the pivotal study, four bone marrow specimens with approximately 0%/100%, 1%/99%, 5%/95% and 95%/5% XY/XX were prepared and analyzed at one site. The mean, standard deviation, and percent CV of the observed percentage of XX and XY nuclei for the bone marrow specimens are shown in Table 1.

Table 1
Precision of the Observed % XY/XX Signaled Nuclei Detection

Specimen Level of XY/XX:		n	Mean (%)		Standard Deviation (%)		Coefficient of Variation (%)	
			XY	XX	XY	XX	XY	XX
0%	100%	10	0.00	97.4	0.00	1.18	—	1.21
1%	99%	20	0.88	97.2	0.48	2.00	54.8	2.06
5%	95%	20	4.90	94.9	0.99	0.99	20.2	1.04
95%	5%	10	95.0	4.96	1.60	1.60	1.68	32.3
99%	1%	24	98.3	0.95	0.41	0.34	0.41	36.3
100%	0%	24	99.0	0.00	0.47	0.00	0.48	—

Methods Comparison: Clinical Specimens

A multi-center, blinded, controlled, comparative study was conducted to characterize the performance of the CEP X/Y DNA probe kit in identifying the proportion of XX and XY cells, relative to standard cytogenetic analysis, in recipients of opposite-sex bone marrow transplants (BMT). Archived bone marrow specimens, which were previously evaluated by standard cytogenetic analysis, were selected from a total of 143 patients 72 males and 71 females), who were the recipients of opposite-sex BMTs. Consecutive specimens were selected and evaluated at three sites; site 1 provided and analyzed 40 specimens; site 2, 52 specimens; and site 3, 51 specimens. These specimens were derived from patients with one of the following diagnoses.

1. Chronic myelogenous leukemia (CML): 69 specimens
2. Acute myeloid leukemia (AML) or Acute nonlymphocytic leukemia (ANLL): 30 specimens
3. Myelodysplastic syndrome (MDS): 7 specimens
4. Acute lymphoid leukemia (ALL): 21 specimens
5. Hematological disorder not otherwise specified, but in which cytogenetics are commonly requested (HDNOS): 16 specimens

All sites utilized unstimulated, cultured specimens for both standard cytogenetic and FISH analyses. Each site followed its own in-house protocol for standard cytogenetic analysis; FISH analyses were performed according to the instructions in the CEP X/Y DNA probe kit package insert. The number of donor and recipient cells were enumerated by FISH in a minimum of 20 metaphase and 500 interphase cells.

As expected for specimens with presumed sex chromosome chimerism after opposite-sex BMT, donor cells were detected in each of the 143 specimens by standard cytogenetic analysis. Interphase FISH analysis designated 143/143 specimens as positive for the presence of donor cells (100% relative sensitivity). FISH metaphase analysis detected donor cells in 141/141[#] specimens (100% relative sensitivity).

In addition to assessing the performance of FISH in the target population of patients with opposite-sex BMT, the ability of interphase and metaphase FISH to correctly designate specimens with like-sex BMT as negative was assessed in 153 patients with like-sex BMTs; the distribution of diagnoses for these patients was similar to those with opposite-sex BMTs. FISH interphase analysis correctly designated 149/153 (97.4%) as negative. All of the four false positive cases occurred in male recipients of like-sex BMT. One case had a 46,XY,-Y,+X karyotype, which led to a FISH result of 37.4% of cells with XX signals; the FISH results of the other three cases showed low levels of XX cells (4.6%, 1.6%, and 0.8%). FISH metaphase analyses designated 151/153 (98.7%) as negative. Both false positive cases were the same patients as those with discrepant FISH interphase analysis. One case had a 46,XY,-Y,+X karyotype, which led to a FISH result of 20% of cells with XX signals; the FISH results of the other case showed 7.1% XX cells.

[#] Two specimens had no metaphase spreads for FISH analysis, thus the total number was 141, instead of 143.

The misclassification of a like-sex BMT recipient with an abnormal acquired karyotype demonstrates the importance of performing pre-BMT cytogenetic analysis in conjunction with FISH. The other 3 false positive cases by FISH had low levels of XX cells; both recipient and donor cells showed a 46,XY karyotype. Although no females with like-sex BMT cases were misclassified by FISH, low levels of donor/recipient cells by FISH should be interpreted with caution. All FISH results should be interpreted in conjunction with standard cytogenetic analysis and within the context of other relevant clinical information.

Conclusions

The performance of CEP X/Y is suppored by the Vysis Quality Control Procedures and is demonstrated in clinical studies. When the CEP X SpectrumOrange / CEP Y SpectrumGreen DNA Probe is used as instructed in the package insert, the above statements describe its performance.